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<b>(21) International Application Number:</b> PCT/FI89/00122 <b>(22) International Filing Date:</b> 27 June 1989 (27.06.89)  <b>(30) Priority data:</b> 883067 27 June 1988 (27.06.88) FI  <b>(71) Applicant (for all designated States except US):</b> LABSYSTEMS OY [FI/FI]; PL 8, SF-00881 Helsinki (FI).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> SAIKKU, Pekka [FI/FI]; LEINONEN, Maija [FI/FI]; Vihdintie 13 A 3, SF-00300 Helsinki (FI).  <b>(74) Agent:</b> RUSKA & CO OY; Runeberginkatu 5, SF-00100 Helsinki (FI).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent),		NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHOD FOR THE TREATMENT AND THE DIAGNOSIS OF CORONARY HEART DISEASE  <b>(57) Abstract</b>  Methods for the treatment and diagnosis of coronary heart disease are described. The invention is based on the foundation that chlamydial infections, especially Chlamydia TWAR infections, play a significant role in the pathogenesis of coronary heart disease. The methods involve drug treatment and screening of or testing for presence of chlamydia or chlamydial antibodies. Also claimed are pharmaceutical preparations of chlamydial infections in coronary heart disease.		

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METHOD FOR THE TREATMENT AND THE DIAGNOSIS OF CORONARY  
HEART DISEASE

FIELD OF THE INVENTION

5        This invention relates to methods and pharmaceuticals to be used in the therapy and prophylaxis of coronary heart disease, as well as to a method for diagnosis and for detecting risks of getting heart infarction.

10      BACKGROUND OF THE INVENTION

      In heart infarction a clog in coronary artery leads to ischemia and partial gangrene of the heart muscle. In angina pectoris the patient suffers from chronich tightness (atherosclerosis) of coronary arteries which lead to  
15      ischemia and to typical pain symptoms. There are several factors which are generally known to increase the risk of getting angina pectoris or heart infarction. These are e.g. high blood pressure, smoking, and low HDL cholesterol content and high LDL cholesterol content of blood.

20      Nowadays the risks of heart infarctions are detected e.g. by measuring total cholesterol and HDL and LDL cholesterol in blood.

      The patients with heart infarctions are nowadays treated e.g. by trombolytic agents, by vasoactive drugs like  
25       $\beta$ -blocking agents, cathecol amines, nitroglycerines, or cardioactive drugs like digitalis. A special risk for the patient is a renewed infarction within a few days from the first infarction. Nowadays this is tried to get prevented primarily by anticoagulants and drugs mentioned above.

30      The prophylaxis of heart infarctions nowadays comprises mainly alteration of dietary and smoking habits, by  $\beta$ -blocking agents and drugs affecting cholesterol metabolism.

      It has also been suggested that circulating or tissue-  
35      deposited immune complexes might play pathogenic role in the development of chronic coronary heart disease and acute myocardial infarction (Füst G, Szondy E, Sekely J, Nanai I, Gerö S, Studies on the occurrence of circulating immune

complexes in vascular diseases, Atherosclerosis 1978:28:181-190; Farrel C, Bloth B, Nielsen H, Dougharty H, Lundman T, Svehag S-E, A survey for circulating immune complexes in patients with acute myocardial infarction, Scand J Immunol 5 1977:6:1233-1240; Cristea A, Rees H, Niculescu F, Bedeleanu D, Vlaicu R, Characterization of circulating immune complexes in heart disease, Immunol Lett, 1986:13:45-49). Immune complexes are thought to act by the activation of complement and thus releasing chemotactic and anaphylactic 10 cleavage products leading to inflammation, platelet aggregation and vasospasm. The presence of immune complexes has been demonstrated in 50 to 60 % of patients after myocardial infarction. However, thus far the antigen in immune complexes has not been characterized except those 15 possibly associated with tissue autoantigens after the injury.

Heart infarction has long been one of the major causes of death. Acute infarction can attack unexpectedly also on relatively young people, which makes the disease especially 20 harmful. Thus there has already long been urgent need of means for screening possible victims of heart infarctions, and for treatment and prophylaxis of coronary heart disease.

#### SUMMARY OF THE INVENTION

25 Now it has been found that chlamydial infections play a significant role in the pathogenesis of coronary heart disease. Especially significant are Chlamydia TWAR infections.

According to the invention heart infarctions can thus 30 often be prevented by treating the possible chlamydial infections. This can be done especially by administering antibiotics which are effective against chlamydia organisms. Chlamydial infections, on the other hand, can be principally prevented by vaccination. For the prevention of heart 35 infarction risks systemic immunity is probably sufficient.

Detection of the possible chlamydial infection can also be used in the therapy of patients who have just undergone a heart infarction. If the possible chlamydial infection is

treated, the risks of getting a new infarction during the recovery can be remarkably decreased. Such a secondary infarction is often highly dangerous.

5 DETAILED DESCRIPTION OF THE INVENTION

Chlamydia are obligatory intracellular gram-negative bacteria. They have been so far divided into two species: C. trachomatis and all the others lumped into a single species of C. psittaci. The last mentioned heterogeneous group also contains so called TWAR strains (Grayston JT, Kuo CC, Wang SP, Altman J, A new Chlamydia psittaci strain, TWAR, isolated in acute respiratory tract infections, N Engl J Med 315:161, 1986). However, quite recently it has been indicated that TWAR strains possess so unique structural and biological characteristics that a status of a completely new chlamydial species has been proposed to them (Kuo CC, Chen HH, Wang SP, Grayston JT, Identification of a new group of Chlamydia psittaci strains called TWAR, J Clin Microbiol 24:1034, 1986; Campbell LA, Kuo CC, Grayston JT, Characterization of the new Chlamydia agent, TWAR, as a unique organism by restriction endonuclease analysis and DNA-DNA hybridization, J Clin Microbiol 25:1911, 1987; Chi EY, Kuo CC, Grayston JT, Unique ultrastructure in the elementary body of Chlamydia sp. strain TWAR, J Bacter 169:3757, 1987; Cox R, Kuo CC, Grayston JT, Campbell LA, Deoxyribonucleic acid relatedness of Chlamydia specis strain TWAR to Chlamydia trachomatis and Chlamydia psittaci, Int J System Bact 38:265, 1988). Chlamydia pneumoniae is the suggested name (Grayston JT, Kuo CC, Campbell LA, Wang SP, Chlamydia pneumoniae sp. nov. for Chlamydia sp. strain TWAR, Int J System Bact 1989:39:88-90).

Studies have revealed the unique nature of TWAR chlamydia. It is difficult to grow, HeLa 229 being the best cell line known so far. Samples should be frozen stepwise for storage at -70 °C. Cultivation demands DEAE-dextran treatment, high centrifugal forces, and incubation at 35 °C in the presence of cycloheximide. The inclusions are stained on the 3rd day with fluorescent monoclonal antibody. In

preliminary screening, group-specific clone can be used, but final proof of an isolate being a TWAR strain demands use of species-specific monoclonal. Adaptation of isolates to cell cultures can be a prolonged process and often demands blind passages in yolk sac, where TWAR strains grow relatively readily, but usually without killing of embryos. Isolates obtained so far have behaved identically in characterization tests despite the differences in time of original isolation or in geographical origin. They possess the common chlamydial lipopolysaccharide (LPS) antigen and are resistant to sulfa drugs, but can easily be separated from C. psittaci by serological methods, and nucleic acid restriction enzyme and hybridization patterns. No plasmid has been found. In electron microscopy they show the typical elementary body (EB) - reticulate body (RB) cycle inside a chlamydial inclusion, but the EB of TWAR chlamydia demonstrates a unique pearshaped structure with a loose outer membrane and a large periplasmic space often containing miniature bodies.

The final criteria justifying the proposal of a new species is supported by the absence of marked DNA homology with the two chlamydial species described earlier.

All chlamydia cause several infectious diseases both in man and animals. C. trachomatis is the causative agent of trachoma in developing countries and is most common cause of genital infections in developed countries. C. psittaci, besides being an important veterinary pathogen, causes respiratory infections in man. TWAR chlamydia has been found out to be a ubiquitous pathogen of humans, and has so far been associated with respiratory tract infections, especially pneumonias (Saikku P, Wang SP, Kleemola M, Brander E, Rusanen E, An epidemic of mild pneumonia due to an unusual strain of Chlamydia psittaci, J Infec Dis 151:832, 1985; Kleemola M, Saikku P, Visakorpi R, Wang SP, Grayston JT, Epidemics of pneumonia caused by TWAR, a new Chlamydia organism, in military trainees in Finland, J Infect Dis 157:230, 1988; Grayston JT; Wang SP, Kuo CC, Altman J, Marrie T, Mordhorst C, Clinical findings in TWAR

respiratory tract infections, In: D Oriel et al, eds.,  
Chlamydial Infections, p 337, Cambridge University Press,  
Cambridge 1986; Marrie TJ, Grayston JT, Wang SP, Kuo CC,  
Pneumonia associated with the TWAR strain of Chlamydia, Ann  
5 Int Med 106:507, 1987; Ekman MR, Saikku P, Kleemola M,  
Visakorpi R, Kuo CC, Grayston JT, Mild and asymptomatic  
respiratory infections with Chlamydia TWAR during a military  
pneumonia epidemic, In: Chlamydia Research I, Proc 1st Symp  
European Soc for Chlamydia Res, p. 56, Almqvist & Wiksell,  
10 Stockholm 1988).

Chlamydia TWAR infections are difficult to diagnose. The  
isolation of organisms by cell culture is not easy to  
perform. At present the most reliable method for the  
etiologial diagnosis of TWAR infections is microimmuno-  
15 fluorescence method for TWAR antibodies (Wang SP, Grayston  
JT, Microimmunofluorescence serological studies with the  
TWAR organism, In "Chlamydial Infections", ed. D. Oriel et  
al. 1986;329-32, Cambridge Univ Press).

By using micro-IF, the role of TWAR strains as  
20 respiratory pathogen has been documented. The complement  
fixation test (CF) and enzymeimmunoassay utilizing Re-LPS,  
shown to be cross-reactive with chlamydial LPS, can be used  
for serological diagnosis of TWAR infections. However, these  
two methods pick up all chlamydial infections, because they  
25 measure antibodies to LPS common to all chlamydial species.

Chlamydia have been recognized as cardiovascular  
pathogens and implicated also in various valvular heart  
diseases. There are reports on myocarditis associated with  
both C. trachomatis (Grayston JT, Mordhorst CH, Wang SP,  
30 Childhood myocarditis associated with Chlamydia trachomatis  
infection, JAMA 1981;246:2823-27; Ringel RE, Brenner JT,  
Rennels MB, Huang SW, Wang SP, Grayston JT, Berman MA,  
Serologic evidence for Chlamydia trachomatis myocarditis,  
Pediatrics 1982;70:54-56) and C. psittaci infections (Dymock  
35 IW, Lawson JM, MacLennan WJ, Ross CAC, Myocarditis  
associated with psittacosis, Brit J Clin Pract 1971;25:240-  
42; Thomas DJB, MacDonald PJ, Fowler JM, Mistaken diagnosis  
- psittacosis myocarditis, Practitioner 1977;218:394-98).

Endocarditis has been demonstrated in connection of infections caused by both species (Editorial, Chlamydial endocarditis, Lancet 1980;i:132; van der Bel-Kahn JM, Watanakunakorn C, Menefee MG, Long HD, Dieter R. Chlamydia trachomatis endocarditis, Amer Heart J 1978;95:627-36) and major arterial emboli caused by C. psittaci have also been described (Bromage D, Jeffries DJ, Philip G, Embolic phenomena in chlamydial infection, J Infection 1980;2:151-59). In addition, even epidemiological association of primary myocardial disease and widespread chlamydial infection has been indicated already in 1967 (Sutton GC, Morrissey RA, Tobin JR, Anderson TO, Pericardial and myocardial disease associated with serological evidence of infection by agents of the psittacosis-lymphogranuloma venerum group (Chlamydiaceae), Circulation 1967;36:830-38; Sutton GC, Demakis JA, Anderson TO, Morrissey RA, Serological evidence of a sporadic outbreak in Illinois of infection by Chlamydia (psittacosis-LGV agent) in patients with primary myocardial disease and respiratory disease, Amer Heart J 1971;81:597-607).

Now it has been found that chlamydia, especially Chlamydia TWAR, are associated also with coronary heart disease and especially with acute infarctions.

The suggested pathogenetic mechanism of Chlamydia TWAR in chronic heart disease and infarction is as follows:

After respiratory infection caused by TWAR, some people develop chronic TWAR infection in which bacteria reside in the cells of reticuloendothelial system either in lungs or heart vascular system. Chlamydia shed their components like (LPS) that are released from infected cells. Antibodies to chlamydial LPS present in blood are bound to circulating LPS forming immunocomplexes and these complexes further increase inflammatory response on vascular endothelium leading to coronary heart disease. In the acute phase of myocardial infarction, antibody levels to LPS in serum are very low suggesting the presence of immunocomplexes and after infarction profound antibody response to LPS can be demonstrated suggesting either liberation of tissue-



deposited immunocomplexes or liberation of antibodies from immunocomplexes or actual response to LPS.

It is further believed that there may be also other still unknown types of chlamydia involved.

5        Thus by the treatment of chronic chlamydial infection by drugs effective against chlamydia chronic coronary heart disease could be cured. On the other hand by the treatment with pharmaceuticals effective against inflammatory effects of chlamydial components myocardial infarction could be  
10       prevented.

      Suitable drugs against chlamydia are e.g. tetracyclines, erythromycin, rifampicillin, and fluoroquinolones. It is not known at present what is the best treatment for chronic  
15       chlamydial infections. Recurrences or reactivation of infections are frequently seen after proper treatment with tetracyclin or erythromycin. Evidently longer treatment (up to four weeks) and higher doses of antibiotics or combination of different antibiotics is needed for  
20       eliminating chronic chlamydial infections.

      Risks of heart infarcts in a patient can also be screened in accordance with the invention. This method comprises taking a sample of blood, sputum, pleural fluid, urine, sample by bronchoscopy or bronchoalveolar lavation or biopsy sample from heart, vascular endothelium, spleen, liver or  
25       lungs and testing the sample for the presence of chlamydia, chlamydial components like LPS or membrane proteins or substances produced by chlamydia like exopolysaccharide or substances produced by host cells by induction of chlamydia or chlamydial antibodies. Suitable techniques are culture  
30       isolation, immunochemical methods like solid phase or liquid immunoassays like enzymeimmunoassay, radioimmunoassay or fluoroimmunoassay, immunofluorescence methods, histological and immunohistochemical staining methods, electronmicroscopy, immunoelectronmicroscopy, or nucleic acid hybrid-  
35       ization. This method can also be used for the diagnosis of myocardial infarction and coronary heart disease, as well as for the monitoring of treatment of patients with acute myocardial infarction or coronary heart disease.

The disease can be e.g. cerebral infarction or vascular embolization.

According to one aspect of the invention vaccine can be prepared and used in the prophylaxis.

5

Association of Chlamydia TWAR with chronic coronary heart disease and myocardial infarction

Patients and controls

10 Patients were divided to two groups. Acute myocardial infarction (AMI) group consisted of 40 consecutive young and middle aged men admitted to the Helsinki University Central Hospital because of an acute myocardial infarction. The inclusion criteria were: male gender (age 50 years or less),  
15 resident in Helsinki or immediate neighbourhood, onset of symptoms within 48 hours prior to the admittance, and verification of acute myocardial infarction by typical changes in electrocardiogram (ECG) and elevation of serum creatinine phosphokinase MB-isoenzyme activity. The mean age  
20 of the patients was 44.5 years (range 34 - 50 years).

Chronic coronary heart disease (CCHD) group consisted of 30 consecutive male patients admitted to the hospital for coronary angiography because of severe chronic symptoms of angina pectoris. Subjects with acute myocardial infarction  
25 within 6 months before entry to the study were excluded. All patients were living in the same area as AMI group and all had chronic coronary heart disease documented both clinically and angiographically. The mean age was 41 years (range 28 to 50 years).

30 Controls were 41 healthy men recruited as a computer based random sample from the official inhabitant records of the city of Helsinki from the same area as patients. Sixty per cent of those invited agreed to participate. The most common cause for refusal was lack of time. None of the  
35 controls had any signs or symptoms of coronary heart disease as judged by negative history and a normal resting ECG. Except for a man who was treated with a beta-blocking agent for hypertension, all the subjects were free of chronic

disease or treatments. The controls were selected to represent the same age range (30 to 50, mean age 39.1 years) as the patients, and they were invited to the examination sequentially one at a time within 2 to 4 weeks after the admission of index case with acute myocardial disease.

Serum samples for determination of antibodies were taken on admission and 4 weeks later from all individuals except for two AMI patients (one succumbed and one dropped out). In addition, 3rd serum sample taken 3 months after admission was obtained from AMI patients.

#### Micro-immunofluorescence (micro-IF) test

A simplified modification of micro-IF assay of WANG & GRAYSTON utilising only TWAR antigen (AR-39, obtained from Washington Research Foundation, Seattle, WA, U.S.A.) was used for determination of chlamydia TWAR antibodies (Wang SP, Grayston JT, Immunologic relationship between genital TRIC, lymphogranuloma venerum, and related organism in a new microtiter indirect immunofluorescence method, Am J Ophthalmol 1970;70:367-74). Fluorescein-isothiocyanate labelled conjugates against human immunoglobulins ( $\lambda$ -chain-specific, Kallestad, Austin, U.S.A.;  $\mu$ -chain-specific, Dakopatt, Denmark;  $\alpha$ -chain-specific, Orion Diagnostics, Finland) were used with amidoschwartz as counterstain. The sera were titrated in two-fold dilutions starting from 1:8, and the limit titer of positivity was considered as 1:32 (Wang SP, Grayston JT, Microimmunofluorescence serological studies with the TWAR organism. In "Chlamydial Infections", ed. D. Oriel et al. 1986;329-32, Cambridge Univ Press).

#### Enzyme-immunoassay for determination of antibodies against chlamydial lipopolysaccharide (LPS-EIA)

Details and validation of the method have been described earlier (Puolakkainen M, Saikku P, Leinonen M, Nurminen M, Mäkelä PH, Chlamydial pneumonitis and its serodiagnosis in infants, J Infec Dis 1984;149:598-604). Briefly, the antigen was LPS from core-deficient mutant of enterobacteria (Re-LPS from Salmonella minnesota, Calbiochem, U.S.A.) shown to

crossreact with chlamydial group antigen (Nurminen M, Leinonen M, Saikku P, Mäkelä PH, The genus-specific antigen of Chlamydiae: Resemblance to the lipopolysaccharide of enteric bacteria, Science 1983;220:1279-81). Alkaline phosphatase labelled conjugates against human IgG, IgM and IgA were obtained from Orion Diagnostics (Espoo, Finland). Titers were expressed as highest dilutions giving OD-value of 0.3.

#### 10 Statistical-analysis

Sample proportions were compared by chi-square test with Yates' correction. When the minimum estimated expected value was under five, Fisher's exact test was used.

#### 15 TWAR IF-antibodies

Both patient groups had significantly more IgG and IgA antibodies than the control group (table 1). The prevalence of IgG antibodies in titers of  $\geq 1:32$  was in AMI group 35/40 ( $p < 0.05$ ) and in CCHD 26/30 ( $p < 0.01$ ) when compared to 24/41 of controls. The respective geometric mean titers (GMT) were 72.5, 76.4 and 24.5. The patient groups had also significantly more elevated IgG titers ( $\geq 128$ ) against the TWAR agent than the controls (20/40 and 14/30 vs. 6/41,  $p < 0.0007$ ). The prevalences of IgA were 11/40 in AMI group and 10/30 in CCHD group vs. 3/41 in controls ( $p < 0.0004$ ), and the GMTs were 15.7, 15.2 and 6.2, respectively. When both markers, elevated IgG and presence of IgA were combined, the significance level was  $p < 0.0001$  (27/40 and 15/30 vs. 7/41 in controls). IgM antibodies were not found.

30

#### EIA antibodies

The majority of patients did not have IgG or IgA antibodies against Re-LPS, and their IgM antibody levels were low when compared to the controls. 26/39 patients with AMI showed a marked IgM antibody response to Re-LPS between first and second serum samples obtained four weeks apart. This response was also seen in IgG and IgA antibodies in somewhat lesser extent. Antibody titers declined in third

serum sample. None of the CCHD patients and only one control showed seroconversion against Re-LPS in their paired serum samples (table 1).

5 Table 1. The presence of elevated IgG or IgA titers against chlamydia TWAR and significant ( $\geq 3$ -fold) seroconversions between paired sera in enzyme immunoassay (EIA) test with Re-LPS antigen in the patient and control groups. AMI = acute myocardial infarction, CCHD = chronic coronary heart  
10 disease.

				Significance	
				AMI vs. CCHD	AMI vs. control
	AMI (No.=40)	CCHD (No.=30)	Control (No.=41)		
TWAR IgG	34	26	25	ns*	0.02
15 $\geq 32$					
TWAR IgG	20	14	6	ns	0.0007
$\geq 128$					
TWAR IgG	78.79	78.79	30.42	ns	0.0002
GMT**					
20 TWAR IgA	18	11	4	ns	0.0004
$\geq 32$					
TWAR IgA	20.39	17.96	10.67	ns	0.0008
GMT					
TWAR IgG/A	27	15	7	ns	0.00001
25 $\geq 128/\geq 32$					
Re-LPS EIA					
$\geq 3$ -fold increase	26/39	0/30	1/41	0.00001	0.00001
* = not significant ( $p > 0.05$ )					
30 ** = geometric mean titer					

Microimmunofluorescence test with TWAR elementary bodies as antigen is a reliable method for measuring specific antibodies to this serologically unique new chlamydial  
35 species (Wang SP, Grayston JT. In "Chlamydial Infections", ed. D. Oriel et al. 1986;329-32, Cambridge Univ Press; Kuo CC, Chen HH, Wang SP, Grayston JT, Identification of a new group of Chlamydia psittaci strains called TWAR, J Clin

Microbiol 1986;24:1034-37). To avoid contamination with tissue lipids present in chlamydial group antigen preparations produced from yolk sacs or tissue cultures, Re-LPS antigen known to share one carbohydrate epitope with chlamydial LPS were used in EIA (Nurminen M, Leinonen M, Saikku P, Mäkelä PH, Science 1983;220:1279-81; Editorial, Psittacosis of non-avian origin, Lancet 1984;ii:442-43). It has also been found that antibody responses to Re-LPS antigen are not found in enterobacterial infections like septicemia and pyelonephritis. Positive antibody responses to Re-LPS have been confirmed to be of chlamydial origin by micro-IF. Thus it is considered that the antibody findings with these different types of test used are diagnostic for chlamydial infection.

Chlamydia TWAR is known to occur in Finland as widespread epidemics, which have conveniently monitored in military settings. Due to these epidemics nearly half of the adult male population in Finland have demonstrable IgG antibodies against chlamydia TWAR, and this is a typical situation in other developed countries as well. In the present study sera were collected in 1983 to 1985. A TWAR epidemic started in Finland in 1985, and the prevalence of low titer ( $\geq 32$ ) antibodies against this chlamydia in 65 % and only a few elevated IgG or IgA antibodies in controls fits well to an antibody status during interepidemic period. However, patients with chronic coronary heart disease and acute myocardial infarction had higher prevalence than expected and significantly higher antibody titers against TWAR chlamydia than the computer matched controls.

Primary TWAR infections are characterized by a predominant IgM response, delayed IgG response and a weak or absent IgA response, whereas secondary infections are characterized with absence of IgM response, and prompt IgG and IgA responses. The patients in the present study had high, stable IgG and IgA titers, and no IgM antibodies in micro-IF test. Altogether, the antibody findings in patients with CCHD and AMI suggest a chronic TWAR chlamydia infection in them. The abnormal responses in Re-LPS EIA further point

to an involvement of chlamydia in coronary heart disease. While not opposing the established risk factors, this unexpected association presents an alternative basis for prophylaxis, treatment and diagnosis of coronary heart disease.

Demonstration of circulating chlamydial LPS immunocomplexes and LPS in patients with myocardial infarction

10 Patients and controls

The AMI patient group consisted of 44 consecutive young, middleaged and old men and women admitted to Helsinki University Central Hospital in 1985 and 1986 because of acute myocardial infarction. The inclusion criteria were: resident in Helsinki or immediate neighbourhood, onset of symptoms within 36 hours prior to the admittance, and verification of acute myocardial infarction by typical changes in ECG and elevation of serum creatinine phosphokinase MB-isoenzyme activity.

20 Controls were 44 healthy men and women recruited as a computer based random sample from official inhabitant records of the city of Helsinki from the same areas as patients. None of the controls had any signs or symptoms of coronary heart disease as judged by negative history and a normal resting ECG. The controls were selected to represent the same age range as the patients and they were invited to the examination sequantially one at the time. Serum samples for immunocomplex determinations were taken on admission and 4 weeks later.

30 The CCHD patient group consisted of 104 young, middle aged and old men and women from Helsinki area whose CCHD was diagnosed by angiography in 1985 and 1986.

Detection of LPS-immunocomplexes

35 A. 96-well EIA-plates were coated with mouse monoclonal antibody to chlamydial LPS. This monoclonal antibody reacts with the KDO-trisaccharide unit specific to chlamydial LPS and not reacting with Re-LPS or Acinetobacter LPS. Serum

samples diluted 1:100 and 1:1000 with PBS containing 10 % fetal calf serum were added into the wells and incubated for 2 hrs at 37 °C. After washing alkaline-phosphatase conjugated anti-human -IgM or -IgG diluted with PBS-fetal calf serum was added and incubated for 2 hours at 37 °C. After washing again the substrate, 1 mg of paranitrophenyl-phosphate in 1 ml of diethanolamine buffer, pH 0.6, was added into the wells and optical density (O.D.) values were measured at 405 nm wavelength after incubation for 30 min. at +37 °C. The amount of chlamydia specific immunocomplexes was expressed at EIA titers: highest serum dilution giving O.D. value of 0.2.

B. 96-well EIA plates were coated with mouse monoclonal antibody against human IgM, ( $\mu$ -chain specific). Serum samples were diluted as in method A were added to the wells. After incubation and washings alkaline-phosphatase conjugated monoclonal antibody to chlamydial LPS was added and EIA procedure was continued as in method A.

## 20 Results

The majority of patients had demonstrable chlamydia LPS - containing immunocomplexes in their sera. The results are shown in Table 2.

25 Table 2. Presence in sera of immunocomplexes reactive with chlamydial LPS specific monoclonal antibody

Ig-class	AMI		Controls	p-value	CCHD
	Patients	p-value			
30 IgM-complexes	24/44 (55 %)	<0.0005	6/44 (14 %)	0.025	35/104 (34 %)
IgG-complexes	4/44 (5 %)	n.s.	1/44 (3 %)	0.0025	26/104 (25 %)
35 Total complexes	26/44 (59 %)	<0.0001	7/44 (16 %)	0.0001	53/104 (51 %)

Detection of chlamydial LPS in patient sera.



Sera were tested for the presence of chlamydia LPS by using commercial chlamydial antigen detection kit for chlamydial LPS (IDEIA<sup>TM</sup>, Boots-Celltech Diagnostics Ltd., Slough, UK), diluted 1 to 8 in dilution buffer included in the kit. LPS was detected in 5/19 (26 %) of AMI patient sera and in none of 19 control sera studied.

The demonstration of chlamydial LPS immune complexes or LPS in AMI patients confirm the association of chlamydial infections with AMI. In addition, the presence of immune complexes further suggest that chlamydial LPS might play important role in the pathogenesis and development of AMI and CCHD.

## CLAIMS:

1. A method for the treatment or prophylaxis of coronary heart disease in a patient, which comprises administering a therapeutically active amount of drug effective against chlamydia or inflammatory effects of chlamydia.
2. A method according to claim 1, wherein the chlamydia is Chlamydia TWAR.
3. A method of screening the risks of heart infarction in a patient, which comprises taking a sample of patient and testing the sample for the presence of chlamydia or chlamydial antibodies.
4. A method to be used for the diagnosis of myocardial infarction and coronary heart disease in a patient, which comprises taking a sample of patient and testing the sample for the presence of chlamydia or chlamydial antibodies.
5. A method to be used for the monitoring of treatment of patients with acute myocardial infarction or coronary heart disease, which comprises taking a sample of patient and testing the sample for the presence of chlamydia or chlamydial antibodies.
6. A method according to any of claims 3 to 5, wherein the sample is tested for the presence of chlamydial saccharides or immune complexes containing chlamydial components.
7. A method according to any of claims 1 to 6, wherein the disease is cerebral infarction or vascular embolization.
8. A pharmaceutical preparation to be used in the treatment or prophylaxis of coronary heart disease in a patient, characterized in that the preparation comprises a therapeutically active amount of drug effective against chlamydia.
9. A method for the preparation of a pharmaceutical preparation to be used in the treatment or prophylaxis of coronary heart disease in a patient, characterized in that a therapeutically active amount drug

effective against chlamydia is mixed in a pharmaceutically acceptable carrier.

10. Vaccine, characterized in that it is used in the prophylaxis of coronary heart disease or myocardial infarction and that it induces immunity against chlamydia.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/FI89/00122

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC 4 <div style="margin-top: 5px;">A 61 K 39/118, 45/06, G 01 N 33/569</div>								
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; margin-top: 5px;">Minimum Documentation Searched 7</div> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 5px;"> <tr> <th style="width: 25%;">Classification System</th> <th style="width: 75%;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">IPC 5</td> <td style="padding: 5px;">A 61 K 39/118; G 01 N 33/569</td> </tr> <tr> <td style="padding: 5px;">US C1</td> <td style="padding: 5px;">424:88; 435:7</td> </tr> </table> <div style="margin-top: 5px; font-size: small;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8</div> <div style="margin-top: 10px;">SE, NO, DK, FI classes as above</div>			Classification System	Classification Symbols	IPC 5	A 61 K 39/118; G 01 N 33/569	US C1	424:88; 435:7
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<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT 9</b> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 5px;"> <tr> <th style="width: 10%;">Category 10</th> <th style="width: 60%;">Citation of Document, 11 with indication, where appropriate, of the relevant passages 12</th> <th style="width: 30%;">Relevant to Claim No. 13</th> </tr> <tr> <td style="vertical-align: top; padding: 5px;">X Y  Y  X,Y    X</td> <td style="vertical-align: top; padding: 5px;">           National Library of Medicine NLM, Database            Medline, accession no. 86081839, "Pericarditis in infective endocarditis", (Ribeiro P. et al.)            Eur Heart J. Nov 1985, 6 (11) p. 975-8.             EP, A2, 0 192 033 (CHIRON CORPORATION ET AL.)            21 August 1986            see the whole document             EP, A2, 0 059 624 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA)            8 September 1982            see the whole document            &amp; JP, 57158725            US, 4427782            CA, 1186223            AU, 549025             National Library of Medicine NLM, Database,            Medline, accession no. 83006426, "Legionnaires disease: a case acquired in Israel", (Isakov A. et al.), Isr J Med Sci Aug 1982, 18 (8) p. 873-7.   <div style="text-align: right;">.../...</div> </td> <td style="vertical-align: top; padding: 5px;">           7-8            3-6, 9, 10               9              3-6                    7, 8         </td> </tr> </table>			Category 10	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13	X Y  Y  X,Y    X	National Library of Medicine NLM, Database Medline, accession no. 86081839, "Pericarditis in infective endocarditis", (Ribeiro P. et al.) Eur Heart J. Nov 1985, 6 (11) p. 975-8.  EP, A2, 0 192 033 (CHIRON CORPORATION ET AL.) 21 August 1986 see the whole document  EP, A2, 0 059 624 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 8 September 1982 see the whole document & JP, 57158725 US, 4427782 CA, 1186223 AU, 549025  National Library of Medicine NLM, Database, Medline, accession no. 83006426, "Legionnaires disease: a case acquired in Israel", (Isakov A. et al.), Isr J Med Sci Aug 1982, 18 (8) p. 873-7.  <div style="text-align: right;">.../...</div>	7-8 3-6, 9, 10    9   3-6         7, 8
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<div style="font-size: x-small;">             * Special categories of cited documents: 14              "A" document defining the general state of the art which is not considered to be of particular relevance              "E" earlier document but published on or after the international filing date              "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)              "O" document referring to an oral disclosure, use, exhibition or other means              "P" document published prior to the international filing date but later than the priority date claimed               "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention              "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step              "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art              "A" document member of the same patent family           </div>								
<b>IV. CERTIFICATION</b> <table border="1" style="width: 100%; border-collapse: collapse; margin-top: 5px;"> <tr> <td style="width: 50%; padding: 5px;">           Date of the Actual Completion of the International Search   <div style="text-align: center;">1989-10-03</div> </td> <td style="width: 50%; padding: 5px;">           Date of Mailing of this International Search Report   <div style="text-align: center;">1989 -10- 0 5</div> </td> </tr> <tr> <td style="width: 50%; padding: 5px;">           International Searching Authority   <div style="text-align: center;">Swedish Patent Office</div> </td> <td style="width: 50%; padding: 5px;">           Signature of Authorized Officer  <div style="text-align: center;">               Carl Olof Gustafsson           </div> </td> </tr> </table>			Date of the Actual Completion of the International Search  <div style="text-align: center;">1989-10-03</div>	Date of Mailing of this International Search Report  <div style="text-align: center;">1989 -10- 0 5</div>	International Searching Authority  <div style="text-align: center;">Swedish Patent Office</div>	Signature of Authorized Officer <div style="text-align: center;">               Carl Olof Gustafsson           </div>		
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	National Library of Medicine NLM, Database Medline, accession no. 88107286, "Combined pericarditis and pneumonia caused by Legionella infection", (Svendsen J.H. et al.), Br Heart J (ENGLAND) Dec 1987, 58 (6) p. 663-4	7, 8
X	National Library of Medicine NLM, Database Medline, accession no. 87100698, "Successful treatment by doxycycline of endocarditis caused by ornithosis", (Walker L.J.), Br Heart J 1987 Jan; 57 (1):58-60	7, 8
X	National Library of Medicine NLM, Database Medline, accession no. 88208346, "In vitro drug susceptibility of Chlamydia sp. strain TWAR., (Kuo C.C.), Antimicrob Agents Chemother 1988 Feb; 32(2):257-8	7, 8